



09 JUN 2005



**(19) World Intellectual Property Organization
International Bureau**

**(43) International Publication Date
24 June 2004 (24.06.2004)**

PCT

(10) International Publication Number
WO 2004/052925 A3

(51) **International Patent Classification⁷:** C12N 15/31,
C07K 14/285, 16/12, C12N 5/10, G01N 33/50, A61K
39/102

Imperial College London, Exhibition Road, London SW7 2AZ (GB).

(21) International Application Number: PCT/GB2003/005349

(72) Inventors; and

(75) **Inventors/Applicants (for US only):** **KROLL, John, Simon [GB/GB]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). LANGFORD, Paul, Richard [GB/GB]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). BOSSE, Janine [CA/CA]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). BEDDEK, Amanda [GB/GB]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). RYCROFT, Andrew [GB/GB]; Imperial College**

[Continued on next page]

(54) Title: ACTINOBACILLUS PLEUROPNEUMONIAE VIRULENCE GENES

(57) Abstract: An attenuated *Actinobacillus pleuropneumoniae* bacterium has a mutation in a gene required for bacterial virulence. Vaccines based upon the bacterium are provided, as are isolated virulence genes and polypeptides and uses thereof.

Class	Strain	Gene	Site/Protein			Known or Putative Function	In Vitro CD	In Vitro OD	
			%	Span	Number				
Cell Surface	BC1	cpnC				AAC20111	chaperone, prokaryotic sigma factor, heat shock	1.628	3.068-03
	2840		leuD (parC)	27030	42	AAP21021	UPE or endopeptidase	1.048	
	987	parU				AMC20011	UPE core transcriptase	1.739	
	2307	parU				AAQ48914	UPE core transcriptase	0.2144	
	48811	parC	parC (par)	103	15	AMQ48914	UPE core transcriptase	0.233	
	1226	parU				AAQ48914	UPE core transcriptase	0.234	
	2188	parP				AMQ48914	UPE core transcriptase	0.233	
	1548	parP	PF0785 (P)	27030	270	SG12877170	UPE core transcriptase	0.735	
	404		parC (par)	24070	20	AMQ48914	transcriptional regulator	1.079	
	1744		parP2 (par)	40250	227	Q42221	Outer membrane protein P2 precursor	1.149	
Membrane	10512		parP (par)	51700	34	PA44810	ADP-ribosyl pyrophosphatase	1.028	
	50411		parP (par)	65000	34	AMC22373	proteobacterial sigma	0.602	
	3527		parA (par)	66000	134	PA42714	ATP synthase	0.214	
	1780		parC (par)	62077	188	PA44810	transmembrane domain	1.160	
	36011		parA (par)	63077	185	PS1310	membrane protein	1.049	3.2584
	583		parA (par)	61040	394	CAA7162	Glycine-tRNA reductase	0.649	
	489		parA (par)	67740	114	PA45311	membrane protein	1.160	
	1805	parP				AMQ48914	ATPase	0.229	
	28911		parC (par)	58070	77	AMQ33981	prokaryotic sigma factor	1.000	1.000
	985		parC (par)	78070	123	AAH48021	prokaryotic sigma phage	1.159	
	647		parB (par)	68070	429	PA4219	NA(DP) cyclase/repressor	0.759	
	2320	parP (par)				AAQ48914	membrane protein, conserved	0.739	
	28410		parC (par)	72061	362	PA42030	prokaryotic sigma factor	0.279	
	28912		parC (par)	61072	21	PA44712	transmembrane protein	1.000	
	28410		parC (par)	68070	185	PA44602	transmembrane	0.607	1.000
	479		parB (par)	64070	258	CAE1510	Glycine tRNA	2.449	3.2584
	27412	parB				Y17101	transmembrane	0.302	1.000
	2805	parC (par)	68050	220	AMQ48914	transmembrane	0.647		
	18411		parC (par)	63050	43	AMQ20110	prokaryotic	0.584	
	2894		parC (par)	76050	137	PA44603	prokaryotic	0.665	
	2307		parC (par)	77050	157	PA44603	prokaryotic RNA	0.744	
	28012		parA (par)	63050	43	PA44520	CSP system	1.000	
Regulatory	8C12		par (par)	76050	120	PTT232	beta galactosidase	0.627	3.2584
	2803		parC (par)	68050	114	PA44707	beta galactosidase	2.003	
	18510		parA (par)	62050	61	AAV028872	negative regulator of cpsE	1.725	0.623
	2103		parC (par)	61050	100	PA44707	beta galactosidase	0.719	0



Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). **SHEEHAN, Brian** [IE/IE]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB).

(74) **Agents:** MACLEAN, Martin, Robert et al.; Mathys & Squire, 100 Gray's Inn Road, London WC1X 8AL (GB).

(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States (regional):** ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) **Date of publication of the international search report:**
4 November 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/05349

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/285 C07K16/12 C12N5/10 G01N33/50
A61K39/102

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 00/61724 A (PHARMACIA & UPJOHN, INC.) 19 October 2000 (2000-10-19) cited in the application the whole document see especially: SEQ ID NOS: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 42 - page 53; examples 7-11; tables 2-4 and page 233 - page 234</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-13, 16-35

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the International filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the International filing date but later than the priority date claimed

- "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

21 June 2004

Date of mailing of the International search report

14 09 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Fuchs, U

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 03/05349

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/075507 A (PHARMACIA & UPJOHN COMPANY) 26 September 2002 (2002-09-26) the whole document see especially: SEQ ID NO: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 45 - page 57; examples 7-11; tables 2-4 and page 234 - page 236 -----	1-13, 16-35
X	ELKINS, C. ET AL.: "Role of the Haemophilus ducreyi Ton System in Internalization of Heme from Hemoglobin" INFECTATION AND IMMUNITY, vol. 66, no. 1, January 1998 (1998-01), pages 151-160, XP002285338 the whole document	16-27, 30-33
A	see especially: page 154 - page 155; figure 2 ExbB, ExbD, TonB proteins and page 158, column 2, line 26 - line 37 -----	1-13, 28, 29, 34, 35
A	FULLER, T.E. ET AL.: "A genetically-defined riboflavin auxotroph of <i>Actinobacillus pleuropneumoniae</i> as a live attenuated vaccine" VACCINE, vol. 18, no. 25, 15 June 2000 (2000-06-15), pages 2867-2877, XP004203577 the whole document -----	1-13, 16-35
T	BEDDEK, A.J. ET AL.: "Two TonB Systems in <i>Actinobacillus pleuropneumoniae</i> : Their Roles in Iron Acquisition and Virulence" INFECTATION AND IMMUNITY, vol. 72, no. 2, February 2004 (2004-02), pages 701-708, XP008031967 the whole document -----	1-13, 16-35

INTERNATIONAL SEARCH REPORT

national application No.
PCT/GB 03/05349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially) because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13, 16-35 (partially)

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)

Present claims 14 and 15 and dependent claims 17, 18, 29-33 relate to a polynucleotide defined by reference to a desirable characteristic or property, namely, for claim 14: "encoding a gene product which is not naturally found in *A. pleuropneumoniae*, but whose expression therein is capable of modulating ... the virulence of that bacterium", and for claim 15: "which is not naturally found in *A. pleuropneumoniae* but which is capable of modulating the virulence of that bacterium by its direct interaction with *A. pleuropneumoniae* virulence genes or gene products".

The claims cover all polynucleotides having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such polynucleotides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the polynucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 14 and 15 and for dependent claims 17, 18, 29-33.

Furthermore, present claims 36, 38-40 relate to an anti-bacterial agent defined by reference to a desirable characteristic or property, namely "identified by the method of claims 34 or 35".

The claims cover all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 36, 38-40.

The same applies to claim 37 relating to a "method of modulating the transcription of such virulence genes through the use of oligonucleotide-directed triplet helix formation". However, the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such oligonucleotides. Accordingly, no search has been carried out for claim 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 16-35 (partially)

An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation in a gene required for bacterial virulence which comprises the nucleotide sequence of SEQ ID NO: 1, a composition containing said attenuated *A. pleuropneumoniae* bacterium, use of said attenuated *A. pleuropneumoniae* bacterium in the manufacture of a medicament for preventing or alleviating an infection of an animal with *A. pleuropneumoniae*, an isolated polynucleotide comprising a) a nucleotide sequence of SEQ ID NO: 1, b) a nucleotide sequence encoding the polypeptide which is encoded by the nucleotide sequence recited in a), c) a nucleotide sequence which hybridizes to the nucleotide sequence of a) and/or b) or to its complement under conditions of moderate to high stringency, d) a fragment of any one of the nucleotide sequences of a)-c) which fragment retains an immunological properties and/or biological activity of the recited nucleotide sequence, a vector comprising said polynucleotide, a host cell comprising said vector, an isolated *A. pleuropneumoniae* polypeptide encoded by said polynucleotide, a method of producing said polypeptide, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide, a method for identifying an anti-bacterial agent which is capable of modulating the function of the said *A. pleuropneumoniae* virulence gene, an attenuated bacterium containing a mutation in a gene comprising a nucleotide sequence which is capable of hybridising to the nucleotide sequence defined by SEQ ID NO: 1 under conditions of moderate to high stringency, a composition containing said attenuated bacterium, use of said attenuated bacterium in the manufacture of a medicament for the therapeutic treatment or prophylactic protection of an animal against infection by the corresponding wild-type bacterium, an isolated virulence polypeptide encoded by said gene, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide;

2. claims: 1-13, 16-35 (partially)

idem as subject 1, but limited to SEQ ID NO: 2;

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/05349

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 0061724	A 19-10-2000		AU 4077600 A BR 0009663 A CA 2366520 A1 CN 1351653 T EP 1171577 A2 JP 2002541790 T NZ 514883 A WO 0061724 A2 US 2004110268 A1 ZA 200108262 A	14-11-2000 09-04-2002 19-10-2000 29-05-2002 16-01-2002 10-12-2002 26-03-2004 19-10-2000 10-06-2004 08-01-2003
WO 02075507	A 26-09-2002		US 2004110268 A1 CA 2438315 A1 EP 1368456 A2 WO 02075507 A2	10-06-2004 26-09-2002 10-12-2003 26-09-2002